Title	<Division of Biochemistry> Biofunctional Design-Chemistry
Author(s)	
Citation	ICR annual report (2007), 13: 20-21
Issue Date	2007-03
URL	http://hdl.handle.net/2433/65539
Right	
Type	Article
Textversion	publisher

Division of Biochemistry
- Biofunctional Design-Chemistry -

http://www.scl.kyoto-u.ac.jp/~bfdc/index.html

Prof
FUTAKI, Shiroh
(D Pharm Sc)

Assist Prof
IMANISHI, Miki
(D Pharm Sc)

Assist Prof
NAKASE, Ikuhiko
(D Pharm Sc)

PD
TADOKORO, Akiko
(D Sc)

Students

TAKEUCHI, Toshihide (D2)	NAKAMURA, Atsushi (M1)	KOBAYASHI, Sachiko (UG)
YAN, Wei (D2)	TAKAYAMA, Kentaro (M1)	KOBAYASHI, Takeshi (UG)
KOSUGE, Michie (M2)	TOHNO, Shunsuke (M1)	NAKAMURA, Yasunori (UG)
MORISAKI, Tatsuya (M2)	HIROSE, Hisaaki (UG)	NOSHIRO, Daisuke (UG)

Visitors

Dr ARWYN, Tomos Jones	Welsh School of Pharmacy, Cardiff University, UK, 13–15 July 2006
Mr BANOCZI, Zoltan	Hungarian Academy of Sciences, Hungary, 23 July–5 August 2006
Prof MATILE, Stefan	University of Geneva, Switzerland, 3 August 2006
Dr SAKAI, Naomi	University of Geneva, Switzerland, 3 August 2006
Prof WOOLLEY, Andrew	University of Toronto, Canada, 3–9 August 2006
Prof GARIEPY, Jean	University of Toronto, Canada, 3–9 August 2006

Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA binding and recognition modes of C2H2-type zinc finger proteins and design of artificial transcription factors with various DNA binding specificities, and (3) design of stimulation-responsible artificial peptides and proteins.

Research Activities (Year 2006)

Presentations

"Membrane-permeable Arginine-rich Peptides and the Interaction with Cell Membranes", Futaki S, 10th Naples Workshop on Bioactive Peptides, Naples, Italy, 11–14 June.

"Oligoarginine Vectors for Intracellular Delivery: Design and Cellular-uptake Mechanisms", Futaki S, The First FIP-APSTJ Joint Workshop on Gene Delivery, Sapporo, 10–12 July.

"Transmission of Extramembrane Conformational Switch into Channel Current; Design and Construction of Artificial Metal-gated Receptor Channel", Kiwada T, Sonomura K, Sugiura Y, Asami K, and Futaki S, International Conference of 43rd Japanese Peptide Symposium/4th Peptide Engineering Meeting, Yokohama, 5–8 November.

"Arginine-rich Peptides and the Internalization Mechanisms", Futaki S, International Mini Symposium Membrane-permeable Peptides: Chemistry, Biology and Therapeutic Applications, Kyoto, 10–11 November.

"Artificial Ion Channels Gating by Extramembrane Conformation Switch", Futaki S, Japan-Italy Research Cooperative Program: Japan-Italy Symposium of New Trends in Enzyme Science and Technology, Nagoya, 15–17 November.

"Creation and Applications of Artificial Zinc Finger-type DNA Binding Proteins", Imanishi M, 55th Annual Meeting, The Japan Society for Analytical Chemistry, Toyonaka, 22 September.

"Selective Modification of N-glycosides of Transferrin

Transmission of Extramembrane Conformational Switch into Channel Current; Design and Construction of Artificial Metal-gated Receptor Channel

Ion channels and receptors are among the most biologically important classes of membrane proteins that transmit outside stimuli into cells. The creation of artificial proteins with these functions is a challenge in peptide/protein engineering in view of the creation of novel functional nanodevices as well as understanding the biological machinery. We have developed a novel Fe(III)-gated ion channel system that is comprised of assemblies of a channel forming peptide alamethicin bearing an extramembrane segment. The extramembrane segment contains a pair of diiminoacetic acid derivatives of lysine (Ida) residues. Interaction with Fe(III) induces the structural alternation of the extramembrane segment via the chelate formation with Ida residues, which eventually leads to an increased channel current (ion influx). This result exemplifies the feasibility of utilizing the conformational switch of the extramembrane segment for the current control in artificial channel systems, a concept that can be applicable for the design of various artificial receptor ion channel systems.

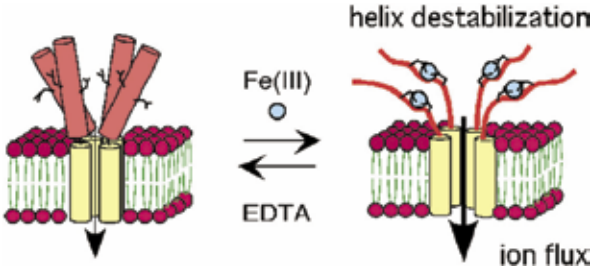

Figure 1. Schematic representation of the artificial receptor channel that transmits outside stimuli (metal) to inside the membrane as an increase in the ion flux.

Direct and Rapid Cytosolic Delivery Using Cell-penetrating Peptides Mediated by Pyrenebutyrate

Intracellular delivery of bioactive molecules using arginine-rich peptides, including oligoarginine and HIV-1 Tat peptides, is a recently developed technology. We found a dramatic change in the methods of internalization for these peptides brought about by the presence of pyrenebutyrate, a counteranion bearing an aromatic hydrophobic moiety. In the absence of pyrenebutyrate, endocytosis plays a major role in cellular uptake. However, the addition of pyrenebutyrate results in direct membrane translocation of the peptides yielding diffuse cytosolic peptide distribution within a few minutes. Using this method, rapid and efficient cytosolic delivery of the enhanced green fluorescent protein (EGFP) was achieved in cells including rat hippocampal primary cultured neurons. Enhancement of bioactivity on the administration of an apoptosis-inducing peptide is also demonstrated. Thus, coupling arginine-rich peptides with this hydrophobic anion dramatically improved their ability to translocate cellular membranes, suggesting the great impact of this approach on exploring and controlling cell function.

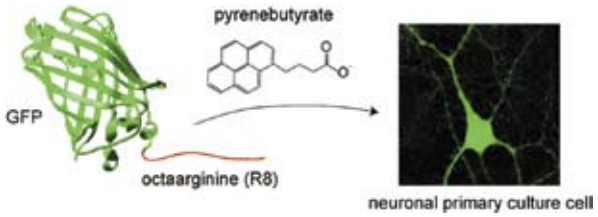

Figure 2. Counteranion-based direct and rapid translocation of R8-conjugated enhanced green fluorescent protein (EGFP) into primary culture cells.

with Therapeutic Drugs for the Receptor Targeting", Nakase I, The First FIP-APSTJ Joint Workshop on Gene Delivery, Sapporo, 10–12 July.

Grants

Futaki S, Development of Intracellular Targeting Vectors and the Real-time Observation of the Intracellular Delivery, Grant-in-Aid for Scientific Research (B), 1 April 2005–31 March 2008.

Futaki S, Cell Targeting Delivery Peptides: Functional Elucidation and Delivery Control, SORST Program, Japan Science and Technology Agency, 1 April 2006–31 March 2008.

Imanishi M, Screening and Evaluation of Novel Clock-related Proteins Using Zinc-finger Technology, PRESTO Program, Japan Science and Technology Agency, 1 October 2005–31 March 2009.

Nakase I, Design and Synthesis of New Carrier Peptides Having Functions of Recognition toward Both Proteoglycans and Cellular Markers for Efficient Delivery of Therapeutic Agents into Cells, Grant-in-Aid for Young Scientists (Start Up), 1 April 2006–31 March 2008.

Award

Nakase I, The Best Presentation Award, The First FIP-APSTJ Joint Workshop on Gene Delivery, Sapporo, 24 July 2006.